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(54) **Title:** PROGNOSIS DETERMINATION IN EWING SARCOMA PATIENTS BY MEANS OF GENETIC PROFILING

(57) **Abstract:** The present invention provides a method for assessing the prognosis of Ewing's Sarcoma patients comprising determining the expression pattern of a defined set of genes in tumor material obtained from said patients, and assigning said expression pattern to either a good prognosis or poor prognosis group.

Prognosis determination in Ewing Sarcoma patients by means of genetic profiling

Field of the Invention

The present invention relates to a method for assessing prognosis in cancer patients. More specifically, the invention disclosed hereinbelow provides a genetic analysis technique that may be used to assess the prognosis of patients with Ewing Sarcoma.

Background of the Invention

Ewing's Sarcoma (ES) is the second most common primary malignant bone tumor in children and adolescents and it belongs to a group of neuroectodermal tumors known as Ewing's Sarcoma Family of Tumors (EFT). This is an aggressive tumor with a high propensity for recurrence and distant metastases [Ginsberg, J.P. *et al.* "Ewing sarcoma family of tumors: Ewing's sarcoma of bone and soft tissue and the peripheral primitive neuroectodermal tumors." *In*: Principles and Practice of Pediatric Oncology, (eds.: Pizzo, P.A. & Poplack) 4th edition, 973-1016, Philadelphia, Pennsylvania, 2002].

All EFT share specific translocations resulting in the fusion of the EWS gene on chromosome 22q12 with different ETS oncogenes on different chromosomes; the most frequent (~95%) is FLI1 on chromosome 11. These translocations are considered distinct diagnostic features of ES tumors

[Delattre, O. *et al.*, *New Eng. J. Med.* 331, 294-299 (1994)].

Both the primary site of the tumor, and the initial response to therapy (assessed histologically as the degree of tumor necrosis following surgery), have become accepted valid prognostic factors in localized tumors. In spite of advances in multimodal therapy, including combination of aggressive chemotherapy, radiotherapy and surgery, about 50% of patients eventually relapse, even after 5 years [Terrier, P. *et al.*, *Semin. Diagn. Pathol.* 13, 250-257 (1996).]

Current clinical and biological characteristics fail to accurately classify ES patients according to their clinical behavior, and it is therefore essential to search for novel reliable prognostic parameters, already at diagnosis.

It is therefore a purpose of the present invention to provide a genetic profiling method for prognosis assessment of patients presenting with ES.

It is another purpose of the invention to provide materials and kits for performing the aforementioned method.

Further objects and advantages of the present invention will become apparent as the description proceeds.

Summary of the Invention

It has now been found that it is possible to distinguish between ES patients having a good prognosis and those having a poor prognosis by means of comparing gene expression patterns in nucleic acid material isolated from the tumors of said patients. Furthermore, it has been found that this prognosis determination may be performed very early on, during initial diagnosis.

The present invention is primarily directed to a method for assessing the prognosis of ES patients comprising determining the expression pattern of a defined set of genes in tumor material obtained from said patients, and assigning said expression pattern to either a good prognosis or poor prognosis group.

The term "good prognosis" is used herein to indicate that the patients are not expected to show ES-related signs, symptoms or evidence for a period of time compatible with the usual clinical meaning of the term. In many cases, this may be taken to mean that the patient is expected to be free from ES-related symptoms for at least five years from assessment. The term "poor prognosis" is similarly used to indicate that the patients are expected to relapse during treatment or within the first few years following treatment.

The term "expression pattern" is used herein to refer to the overall profile of results obtained when the expression of a defined set of genes is determined. Such a pattern is advantageous since it facilitates the use of both quantitative, statistical analytical techniques as well as permitting rapid visual inspection and comparison of results. Preferably (but not exclusively) such a pattern is obtained by the use of a matrix method, such as a high density microarray method.

Although any suitable technique may be used to determine the expression of the aforementioned defined set of genes, in one preferred embodiment of the method, this technique is a nucleic acid hybridization technique.

In a particularly preferred embodiment, the nucleic acid hybridization technique comprises the steps of extracting total RNA from the ES-patient tumor material, generating double-stranded cDNA from said total RNA, performing *in vitro* transcription of said cDNA, labeling the RNA transcript obtained thereby, preparing a hybridization mix comprising said labeled RNA transcript together with irrelevant and control nucleic acid sequences, hybridization of said hybridization mix to a solid-state human genome microarray and generating and amplifying a hybridization signal. This hybridization signal provides a visual expression pattern which may then be assigned to one of the good or poor prognosis groups.

In another preferred embodiment, the hybridization technique used is selected from the group consisting of northern blotting and western blotting.

In other preferred embodiments of the invention, gene expression may be determined by the use of a technique other than a hybridization technique. In a particularly preferred embodiment, the technique is selected from the group consisting of RT-PCR, semi-quantitative RT-PCR, quantitative real time RT-PCR, immunohistochemistry and ELISA.

In one particularly preferred embodiment of the method of the invention, the assignment of the gene expression pattern to one of the good or poor prognosis groups is performed by means of a hierarchical clustering technique.

In one preferred embodiment of the method of the invention, the aforementioned defined set of genes comprises genes selected from the group of 818 genes listed in table 1, hereinbelow.

In another preferred embodiment, the defined set of genes consists of between 1 and 100 genes selected from the aforementioned group of 818 genes.

In another preferred embodiment, the defined set of genes consists of between 101 and 200 genes selected from the aforementioned group of 818 genes.

In another preferred embodiment, the defined set of genes consists of between 201 and 300 genes selected from the aforementioned group of 818 genes.

In another preferred embodiment, the defined set of genes consists of between 301 and 400 genes selected from the aforementioned group of 818 genes.

In another preferred embodiment, the defined set of genes consists of between 401 and 500 genes selected from the aforementioned group of 818 genes.

In another preferred embodiment, the defined set of genes consists of between 501 and 600 genes selected from the aforementioned group of 818 genes.

In another preferred embodiment, the defined set of genes consists of between 601 and 700 genes selected from the aforementioned group of 818 genes.

In another preferred embodiment, the defined set of genes consists of between 701 and 818 genes selected from the aforementioned group of 818 genes.

In another aspect, the present invention is also directed to a solid-state nucleic acid microarray comprising at least two nucleic acids affixed to a substrate, wherein each of said at least two nucleic acids consists of a

partial sequence of one of the genes present in the aforementioned group of 818 genes.

In one preferred embodiment, the microarray of the present invention comprises between 2 and 100 nucleic acid sequences, wherein each of said sequences consists of a partial sequence of one of the genes present in the aforementioned group of 818 genes.

In another preferred embodiment, the microarray of the present invention comprises between 101 and 200 nucleic acid sequences, wherein each of said sequences consists of a partial sequence of one of the genes present in the aforementioned group of 818 genes.

In another preferred embodiment, the microarray of the present invention comprises between 201 and 300 nucleic acid sequences, wherein each of said sequences consists of a partial sequence of one of the genes present in the aforementioned group of 818 genes.

In another preferred embodiment, the microarray of the present invention comprises between 301 and 400 nucleic acid sequences, wherein each of said sequences consists of a partial sequence of one of the genes present in the aforementioned group of 818 genes.

In another preferred embodiment, the microarray of the present invention comprises between 401 and 500 nucleic

acid sequences, wherein each of said sequences consists of a partial sequence of one of the genes present in the aforementioned group of 818 genes.

In another preferred embodiment, the microarray of the present invention comprises between 501 and 600 nucleic acid sequences, wherein each of said sequences consists of a partial sequence of one of the genes present in the aforementioned group of 818 genes.

In another preferred embodiment, the microarray of the present invention comprises between 601 and 700 nucleic acid sequences, wherein each of said sequences consists of a partial sequence of one of the genes present in the aforementioned group of 818 genes.

In another preferred embodiment, the microarray of the present invention comprises between 701 and 818 nucleic acid sequences, wherein each of said sequences consists of a partial sequence of one of the genes present in the aforementioned group of 818 genes.

In a particularly preferred embodiment, the microarray of the present invention comprises all of the 818 genes present in the aforementioned group of genes.

In addition to the aforementioned at least two nucleic acids, the microarray may also comprise one or more control nucleic acid sequences.

The substrate present in the microarray may consist of any suitable material or combination of materials. Preferably, however, the substrate is selected from the group consisting of ceramics, glasses, metal oxides, nitrocellulose and nylon.

In a further aspect, the present invention also provides a kit comprising a solid-state nucleic acid microarray as defined and described herein together with an instruction sheet.

Kits based on the other gene expression technologies used in the method of the invention (as described hereinabove) are also within the scope of the present invention. Thus, in one embodiment, the kit of the present invention comprises a set of relevant primers suitable for use in real time RT-PCR together with control solutions and an instruction sheet. In another embodiment, the kit comprises micro-well plates or similar vessels suitable for use in an ELISA assay, together with antibodies specific for isotopes present on the peptides and polypeptides expressed from the aforementioned defined set of genes, suitable reagents for signal detection and amplification and an instruction sheet. In yet another embodiment, the kit comprises antibodies specific for isotopes present on the peptides and polypeptides expressed from the aforementioned defined set of genes, together with reagents

suitable for signal detection and amplification using standard immunochemical methods and an instruction sheet.

All the above and other characteristics and advantages of the present invention will be further understood from the following illustrative and non-limitative examples of preferred embodiments thereof.

Brief Description of the Drawings

Fig. 1 illustrates the hierarchical clustering, Kaplan-Meier PFS analysis and gene clusters of Ewing sarcoma tumor samples.

a, Illustration of the two sided clusters dendogram, distinctly defining poor prognosis (1st 8 columns from left to right) vs. good prognosis (6 right-most columns) groups of ES patients and the differentially expressed genes. Each column represents a patient and each row represents a gene.

b, Kaplan-Meier progression free survival analysis presents a significant correlation between poor prognosis vs. good prognosis patients, according to the microarray classification.

c, The 2 major gene clusters and the 6 subclusters, formed on the basis of the similarities of the 818 genes measured over the 14 tumor samples. The 2 gene clusters consist of differentially expressed genes: over-expressed in the poor

prognosis group and down-regulated in the good prognosis group, and vice versa.

Fig. 2 graphically illustrates the correlation between expression of the cadherin-11 and the MTA1 genes by microarray analysis and by Real Time PCR.
a, Expression mean log value of cadherin-11 in poor prognosis patients was significantly higher than the expression mean value in good prognosis patients by both analyses.
b, Gene expression pattern in the poor and good prognosis patients, was also significantly correlated by both analyses, for the MTA1 gene.

Detailed Description of Preferred Embodiments

As mentioned, hereinabove, ES is the second most common primary malignant bone tumor in children and adolescents. In spite of advances in multimodal therapy, about 50% of patients eventually relapse, even after 5 years or more. Currently accepted clinical prognostic factors, fail to classify ES patients' risk to relapse at diagnosis.

The recent development of DNA microarrays provides an opportunity to take a genome-wide approach to extend biological insights into all aspects of the study of disease: pathogenesis, disease development, staging, prognosis and treatment response. Gene expression profiling using oligonucleotide high-density arrays has provided an

additional tool for elucidating tumor biology as well as the potential for molecular classification of cancer.

In the method of the present invention, oligonucleotide high-density array analysis of material derived from primary tumors is used to identify two distinct gene expression profiles distinguishing ES patients with poor and good prognosis. The results obtained with this method (including the results presented in the Example hereinbelow) indicate the existence of a specific gene expression signature of outcome in ES, already at diagnosis thereby providing a strategy, based upon gene expression patterns, for selecting patients who would benefit from risk adapted improved therapy. The gene expression patterns used in this strategy are based on data sets containing a minimum of 1 significant gene out of the 818 genes to a maximum of 818 genes. Intermediate-sized datasets containing up to 100 genes, 200 genes, 300 genes, 400 genes, 500 genes, 600 genes, 700 genes and 800 genes, may also be usefully defined and used in said selection and prognostic strategy. The present invention also encompasses nucleic acid bearing microarrays for use in the method disclosed herein, as well as kits containing all of the necessary materials and instructions for performing the abovementioned strategy or method, as disclosed and described in more detail hereinbelow.

The details of the aforementioned group of 818 genes for use in accordance with a particularly preferred embodiment of the present invention are listed in Table 1:

Table 1

Gene	Gene Name	GeneBank ID
FLII	flightless I homolog (Drosophila)	U80184
PM5	pM5 protein	X57398
PBEF	pre-B-cell colony-enhancing factor	U02020
KIAA0892	KIAA0892 protein	AB020699
HSD17B4	hydroxysteroid (17-beta) dehydrogenase 4	X87176
IGKC	immunoglobulin kappa constant	X96754
CDC14B	CDC14 cell division cycle 14 homolog B (S. cerevisiae)	AI739548
SLC22A6	"solute carrier family 22 (organic anion transporter), member 6"	AB009698
NRTN	neurturin	U78110
KIAA1096	KIAA1096 protein	AL096857
IFRD1	interferon-related developmental regulator 1	AC005192
KIAA0310	KIAA0310 gene product	AB002308
ACAA1	acetyl-Coenzyme A acyltransferase 1 (peroxisomal 3-oxoacyl-Coenzyme A thiolase)	X14813
GRN	granulin	AF055008
SH3BGR	SH3 domain binding glutamic acid-rich protein	X93498
MJD	"Machado-Joseph disease (spinocerebellar ataxia 3, olivopontocerebellar ataxia 3, autosomal dominant, ataxin 3)"	U64820
DKFZP564G2022	DKFZP564G2022 protein	AL049944
EWSR1	Ewing sarcoma breakpoint region 1	X66899
AHCYL1	S-adenosylhomocysteine hydrolase-like 1	AI800578
KLRC3	"killer cell lectin-like receptor subfamily C, member 3"	AJ001685
F2RL1	coagulation factor II (thrombin) receptor-like 1	U34038
EIF4G1	"eukaryotic translation initiation factor 4 gamma, 1"	D12686
		D26561
TP53BP2	"tumor protein p53 binding protein, 2"	U58334

TP63	tumor protein p63	Y16961
MAN2B1	"mannosidase, alpha, class 2B, member 1"	U60899
BLCAP	bladder cancer associated protein	AL049288
TAF6	"TAF6 RNA polymerase II, TATA box binding protein (TBP)-associated factor, 80kDa"	L25444
	H.sapiens hsr1 mRNA (partial)	X66436
STRN3	"striatin, calmodulin binding protein 3"	U17989
KIAA0914	KIAA0914 gene product	AB020721
SYNE-2	synaptic nuclei expressed gene 2	AL080133
LLGL1	lethal giant larvae homolog 1 (Drosophila)	X86371
		M62302
PSMD9	"proteasome (prosome, macropain) 26S subunit, non-ATPase, 9"	AB003177
IL4	interleukin 4	M13982
EP400	E1A binding protein p400	AI143868
DPAGT1	dolichyl-phosphate (UDP-N-acetylglucosamine) N-acetylglucosaminephosphotransferase 1 (GlcNAc-1-P transferase)	Z82022
MKNK1	MAP kinase-interacting serine/threonine kinase 1	AB000409
KIAA0356	KIAA0356 gene product	AB002354
MET	met proto-oncogene (hepatocyte growth factor receptor)	J02958
TPO	thyroid peroxidase	J02969
EGFL5	"EGF-like-domain, multiple 5"	AB011542
RRS1	homolog of yeast ribosome biogenesis regulatory protein RRS1	D25218
ARL1	ADP-ribosylation factor-like 1	L28997
SDCBP	syndecan binding protein (syntenin)	AF000652
B7	B7 protein	U72508
SDBCAG84	serologically defined breast cancer antigen 84	AF091085
	Homo sapiens mRNA; cDNA DKFZp434M162 (from clone DKFZp434M162)	W72239
REL	v-rel reticuloendotheliosis viral oncogene homolog (avian)	AA872560
SEMA3F	"sema domain, immunoglobulin domain (Ig), short basic domain, secreted, (semaphorin) 3F"	U38276
		X71346
KLK3	"kallikrein 3, (prostate specific antigen)"	X07730
F7	coagulation factor VII (serum prothrombin conversion accelerator)	M13232
RBBP2	retinoblastoma binding protein 2	S66431
KIAA0020	KIAA0020 gene product	D13645
GRIN2A	"glutamate receptor, ionotropic, N-methyl D-aspartate 2A"	U09002

GART	"phosphoribosylglycinamide formyltransferase, phosphoribosylglycinamide synthetase, phosphoribosylaminoimidazole synthetase"	X54199
PSMB8	"proteasome (prosome, macropain) subunit, beta type, 8 (large multifunctional protease 7)"	X87344
HTR2A	5-hydroxytryptamine (serotonin) receptor 2A	AA418537
SURB7	SRB7 suppressor of RNA polymerase B homolog (yeast)	U52960
MAP3K7IP2	mitogen-activated protein kinase kinase kinase 7 interacting protein 2	AB018276
MGST3	microsomal glutathione S-transferase 3	AF026977
PFDN1	prefoldin 1	D45333
U2AF65	U2 small nuclear ribonucleoprotein auxiliary factor (65kD)	AI762438
KRTHA2	"keratin, hair, acidic, 2"	X90761
POU4F1	"POU domain, class 4, transcription factor 1"	L20433
CTSO	cathepsin O	AI810485
MAPK9	mitogen-activated protein kinase 9	U09759
ISLR	immunoglobulin superfamily containing leucine-rich repeat	AB003184
DKFZP566B183	DKFZP566B183 protein	AL050272
USP24	ubiquitin specific protease 24	AB028980
PBX2	pre-B-cell leukemia transcription factor 2	X59842
HT012	uncharacterized hypothalamus protein HT012	AI760162
		X17360
		HG162-HT3165
HRIHFB2206	HRIHFB2206 protein	L10379
SYBL1	synaptobrevin-like 1	X92396
GRM4	"glutamate receptor, metabotropic 4"	X80818
ATP5H	"ATP synthase, H+ transporting, mitochondrial F0 complex, subunit d"	AF087135
MGC5149	hypothetical protein MGC5149	U79260
C20orf188	chromosome 20 open reading frame 188	AF055022
ZNF238	zinc finger protein 238	U38896
KIAA1030	KIAA1030 protein	AB028953
PLU-1	putative DNA/chromatin binding motif	AJ132440
CCT8	"chaperonin containing TCP1, subunit 8 (theta)"	D13627
XRCC2	X-ray repair complementing defective repair in Chinese hamster cells 2	Y08837
KIAA0170	KIAA0170 gene product	AL041663
LPIN2	lipin 2	D87436

SULT4A1	"sulfotransferase family 4A, member 1"	W25958
CDX2	caudal type homeo box transcription factor 2	U51096
CFDP1	craniofacial development protein 1	D85939
CDK2	cyclin-dependent kinase 2	HG1155-HT4822 M68520
KIAA0737	KIAA0737 gene product	AF014837
NTSR2	neurotensin receptor 2	Y10148
PRSS15	"protease, serine, 15"	X76040
UBE2M	"ubiquitin-conjugating enzyme E2M (UBC12 homolog, yeast)"	AF075599
NEUROD2	neurogenic differentiation 2	AB021742
PCBP3	poly(rC) binding protein 3	AL046394
CDK5	cyclin-dependent kinase 5	L04658
UBE3B	ubiquitin protein ligase	AL096740
ALDH9A1	"aldehyde dehydrogenase 9 family, member A1"	U34252
HCS	cytochrome c	D00265
TUFM	"Tu translation elongation factor, mitochondrial"	S75463
TFCP2	transcription factor CP2	U03494
KIAA0963	KIAA0963 protein	AI760801
SIAH1	seven in absentia homolog 1 (Drosophila)	W26406
CRHR2	corticotropin releasing hormone receptor 2	AF011406
SLC7A11	"solute carrier family 7, (cationic amino acid transporter, y+ system) member 11"	AB026891
COL6A1	"collagen, type VI, alpha 1"	AA885106
PTENP1	"phosphatase and tensin homolog (mutated in multiple advanced cancers 1), pseudogene 1"	AF019083
PDAP1	PDGFA associated protein 1	U41745
RAD50	RAD50 homolog (S. cerevisiae)	U05681 U63139
LRBA	"LPS-responsive vesicle trafficking, beach and anchor containing"	M13970 M83822
ARS2	arsenate resistance protein ARS2	AI972631 AJ002428
ANXA2P1	annexin A2 pseudogene 1	M62896
ERCC2	"excision repair cross-complementing rodent repair deficiency, complementation group 2 (xeroderma pigmentosum D)"	AA079018
ORC3L	"origin recognition complex, subunit 3-like (yeast)"	AL080116
TNFRSF12	"tumor necrosis factor receptor superfamily, member 12 (translocating chain-association membrane protein)"	U83598
COX6A1	cytochrome c oxidase subunit VIa polypeptide 1	AI540925

PRL	prolactin	M29386
PIM1	pim-1 oncogene	M54915
	Homo sapiens mRNA full length insert cDNA clone EUROIMAGE 42138	AL109702
CCBP2	chemokine binding protein 2	U94888
PTS	6-pyruvoyltetrahydropterin synthase	L76259
GSTA4	glutathione S-transferase A4	AF025887
PRSS25	"protease, serine, 25"	AF020760
SEC14L1	SEC14-like 1 (S. cerevisiae)	D67029
FGF18	fibroblast growth factor 18	AA022949
		U46194
FLJ20580	hypothetical protein FLJ20580	AI862521
DKFZP586B0923	DKFZP586B0923 protein	AL050190
	Homo sapiens mRNA; cDNA DKFZp434A012 (from clone DKFZp434A012)	AL096752
PTK2B	protein tyrosine kinase 2 beta	U43522
RNF13	ring finger protein 13	AF037204
ATR	ataxia telangiectasia and Rad3 related	U49844
USP19	ubiquitin specific protease 19	AB020698
DDX21	DEAD/H (Asp-Glu-Ala-Asp/His) box polypeptide 21	U41387
STK3	"serine/threonine kinase 3 (STE20 homolog, yeast)"	U26424
MAAT1	melanoma-associated antigen recognised by cytotoxic T lymphocytes	U19796
		W28193
TMEM1	transmembrane protein 1	AB001523
MYB	v-myb myeloblastosis viral oncogene homolog (avian)	M13666
RER1	similar to S. cerevisiae RER1	AW044624
RBM9	RNA binding motif protein 9	AA402524
DKFZP586A0522	DKFZP586A0522 protein	AL050159
MVK	mevalonate kinase (mevalonic aciduria)	M88468
CHIT1	chitinase 1 (chitotriosidase)	U29615
	"Homo sapiens cDNA FLJ32313 fis, clone PROST2003232, weakly similar to BETA-GLUCURONIDASE PRECURSOR (EC 3.2.1.31)"	AI932613
KIAA1079	KIAA1079 protein	AI971726
TCFL4	transcription factor-like 4	AW005997
UBE2B	ubiquitin-conjugating enzyme E2B (RAD6 homolog)	M74525
HR44	Hr44 antigen	X91103
CDC5L	CDC5 cell division cycle 5-like (S. pombe)	AB007892
EIF4G1	"eukaryotic translation initiation factor 4 gamma, 1"	AF104913
GNB1	"guanine nucleotide binding protein (G protein), beta polypeptide 1"	X04526
NRG2	neuregulin 2	AA706226

XPNPEP1	"X-prolyl aminopeptidase (aminopeptidase P) 1, soluble"	X95762
ODC1	ornithine decarboxylase 1	X16277
ALMS1	Alstrom syndrome 1	R40666
VAPB	VAMP (vesicle-associated membrane protein)-associated protein B and C	W27026
UTRN	utrophin (homologous to dystrophin)	X69086
GPR49	G protein-coupled receptor 49	AF062006
PPP2R4	"protein phosphatase 2A, regulatory subunit B' (PR 53)"	X73478
RABGGTB	"Rab geranylgeranyltransferase, beta subunit"	X98001
AP3S2	"adaptor-related protein complex 3, sigma 2 subunit"	X99459
KIAA0171	KIAA0171 gene product	D79993
ABCC8	"ATP-binding cassette, sub-family C (CFTR/MRP), member 8"	L78207
LOC51634	CGI-79 protein	AL050405
	Homo sapiens clone 24487 mRNA sequence	AF070579
SAH	SA hypertension-associated homolog (rat)	X80062
TCF8	transcription factor 8 (represses interleukin 2 expression)	U19969
ADCYAP1	adenylate cyclase activating polypeptide 1 (pituitary)	X60435
DEK	DEK oncogene (DNA binding)	X64229
DBP	D site of albumin promoter (albumin D-box) binding protein	U48213
ITGAE	"integrin, alpha E (antigen CD103, human mucosal lymphocyte antigen 1; alpha polypeptide)"	L25851
ABCF2	"ATP-binding cassette, sub-family F (GCN20), member 2"	AJ005016
SC5DL	"sterol-C5-desaturase (ERG3 delta-5-desaturase homolog, fungal)-like"	AB016247
		D50525
LGALS9	"lectin, galactoside-binding, soluble, 9 (galectin 9)"	Z49107
CUL1	cullin 1	U58087
GYPE	glycophorin E	X53004
DIAPH2	diaphanous homolog 2 (Drosophila)	Y15909
PSR	phosphatidylserine receptor	AI950382
LIPA	"lipase A, lysosomal acid, cholesterol esterase (Wolman disease)"	X76488
PSMD11	"proteasome (prosome, macropain) 26S subunit, non-ATPase, 11"	AB003102
PSMA3	"proteasome (prosome, macropain) subunit, alpha type, 3"	D00762
VBP1	von Hippel-Lindau binding protein 1	U56833
SIX6	sine oculis homeobox homolog 6 (Drosophila	AJ011785
RBL2	retinoblastoma-like 2 (p130)	X76061
KCNAB1	"potassium voltage-gated channel, shaker-related subfamily, beta member 1"	X83127

EP300	E1A binding protein p300	U01877
ABO	"ABO blood group (transferase A, alpha 1-3-N-acetylgalactosaminyltransferase; transferase B, alpha 1-3-galactosyltransferase)"	X84746
GRIK5	"glutamate receptor, ionotropic, kainate 5"	AA977136
ADPRTL1	ADP-ribosyltransferase (NAD+; poly (ADP-ribose) polymerase)-like 1	AF057160
HBXIP	hepatitis B virus x interacting protein	AF029890
BHC80	BRAF35/HDAC2 complex (80 kDa)	W25985
KIAA0436	putative L-type neutral amino acid transporter	AB007896
MDH2	"malate dehydrogenase 2, NAD (mitochondrial)"	AF047470
KIAA0630	KIAA0630 protein	AB014530
IL1RL1	interleukin 1 receptor-like 1	D12763
DMTF1	cyclin D binding myb-like transcription factor 1	AF052102
MLH1	"mutL homolog 1, colon cancer, nonpolyposis type 2 (E. coli)"	U07418
GGTLA1	gamma-glutamyltransferase-like activity 1	M64099
FHIT	fragile histidine triad gene	U46922
ZNF278	"ESTs, Weakly similar to I38724 mitochondrial benzodiazepine receptor - human [H.sapiens]"	AI052224
	zinc finger protein 278	AI352450
HLCS	holocarboxylase synthetase (biotin-[proprionyl-Coenzyme A-carboxylase (ATP-hydrolysing)] ligase)	D87328
LOC57147	hypothetical protein LOC57147	W26641
HTR4	5-hydroxytryptamine (serotonin) receptor 4	Y12505
MORF	monocytic leukemia zinc finger protein-related factor	AB002381
AANAT	arylalkylamine N-acetyltransferase	U40391
MGP	matrix Gla protein	AI953789
		AB012229
FLJ13052	NAD kinase	AL031282
VAPB	VAMP (vesicle-associated membrane protein)-associated protein B and C	W25933
ENTPD1	ectonucleoside triphosphate diphosphohydrolase 1	AJ133133
SDF2	stromal cell-derived factor 2	D50645
		U60269
KIAA0907	KIAA0907 protein	AB020714
SPRR2C	small proline-rich protein 2C	M21539
DNAJB5	"DnaJ (Hsp40) homolog, subfamily B, member 5"	AF088982
FMR2	fragile X mental retardation 2	U48436
SLC7A8	"solute carrier family 7 (cationic amino acid transporter, y+ system), member 8"	Y18483
E2F5	"E2F transcription factor 5, p130-binding"	U31556
LSM3	Lsm3 protein	N98670
FLJ22678	hypothetical protein FLJ22678	AA165701

PRKCABP	"protein kinase C, alpha binding protein"	AL049654
DIP2	disco-interacting protein 2 (Drosophila) homolog	D80006
CEP1	centrosomal protein 1	AF083322
PAX6	"paired box gene 6 (aniridia, keratitis)"	M93650
HLALS	"major histocompatibility complex, class I-like sequence"	AF031469
MPV17	"MpV17 transgene, murine homolog, glomerulosclerosis"	X76538 W29045
KIAA0217	KIAA0217 protein	D86971
RANBP7	RAN binding protein 7	AF098799
UBE4A	"ubiquitination factor E4A (UFD2 homolog, yeast)"	D50916
KIAA0337	KIAA0337 gene product	AB002335
UPK1A	uroplakin 1A	AF085807
ELAVL2	"ELAV (embryonic lethal, abnormal vision, Drosophila)-like 2 (Hu antigen B)"	U29943
PISD	phosphatidylserine decarboxylase	AL050371
ZP3A	zona pellucida glycoprotein 3A (sperm receptor)	X56777
HDAC3	histone deacetylase 3	U75697
AD024	AD024 protein	W28510
PFKFB2	"6-phosphofructo-2-kinase/fructose-2,6-biphosphatase 2"	AJ005577
RRH	retinal pigment epithelium-derived rhodopsin homolog	AF012270
IGHMBP2	immunoglobulin mu binding protein 2	L14754
DSPG3	dermatan sulfate proteoglycan 3	U59111
	Homo sapiens mRNA; cDNA DKFZp434M245 (from clone DKFZp434M245)	W28661
MAPK9	mitogen-activated protein kinase 9	U09759 U64871
AMMECR1	"Alport syndrome, mental retardation, midface hypoplasia and elliptocytosis chromosomal region, gene 1"	AJ007014
ATP6V1D	"ATPase, H+ transporting, lysosomal 34kDa, V1 subunit D"	AA877795
ANP32A	"acidic (leucine-rich) nuclear phosphoprotein 32 family, member A"	U73477
PFAS	phosphoribosylformylglycinamidine synthase (FGAR amidotransferase)	AB002359
CPNE3	copine III	AB014536
KIAA0410	KIAA0410 gene product	AB007870
SET	SET translocation (myeloid leukemia-associated)	M93651
CSTF2	"cleavage stimulation factor, 3' pre-RNA, subunit 2, 64kDa"	M85085
ASNA1	"arsA arsenite transporter, ATP-binding, homolog 1 (bacterial)"	AF047469
SLC2A1	"solute carrier family 2 (facilitated glucose transporter), member 1"	K03195
C8orf1	chromosome 8 open reading frame 1	AI738702

	Homo sapiens mRNA; cDNA DKFZp586K2322 (from clone DKFZp586K2322)	AL080113
TM9SF1	transmembrane 9 superfamily member 1	U94831
NDP	Norrie disease (pseudoglioma)	X65724
YWHAE	"tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, epsilon polypeptide"	U54778
KCNJ6	"potassium inwardly-rectifying channel, subfamily J, member 6"	U52153
		X03453
RFPL3	ret finger protein-like 3	AJ010232
HCFC1	host cell factor C1 (VP16-accessory protein)	U52112
SLC12A4	"solute carrier family 12 (potassium/chloride transporters), member 4"	AF054506
T	"T, brachyury homolog (mouse)"	AJ001699
ZNF174	zinc finger protein 174	U31248
TRAP100	thyroid hormone receptor-associated protein (100 kDa)	D50920
HTR6	5-hydroxytryptamine (serotonin) receptor 6	L41147
NASP	nuclear autoantigenic sperm protein (histone-binding)	M97856
COMT	catechol-O-methyltransferase	M58525
AXL	AXL receptor tyrosine kinase	M76125
NME1	"non-metastatic cells 1, protein (NM23A) expressed in"	X73066
		M10098
LOC51055	unknown	U88048
CREM	cAMP responsive element modulator	S68271
MEF-2	myelin gene expression factor 2	W28567
PCBP1	poly(rC) binding protein 1	Z29505
GNG5	"guanine nucleotide binding protein (G protein), gamma 5"	AI541042
CNNM2	cyclin M2	AI827730
NCSTN	nicastrin	D87442
ICOS	inducible T-cell co-stimulator	AB023135
TK2	"thymidine kinase 2, mitochondrial"	U80628
LTK	leukocyte tyrosine kinase	X52213
BRD2	bromodomain containing 2	D42040
SMAP	skeletal muscle abundant protein	AF016270
	Homo sapiens retinoic acid-inducible endogenous retroviral DNA	M64936
MYO1C	myosin IC	X98507
IMAGE145052	small acidic protein	AI346580
	"AML1=AML1 (alternatively spliced, exons 5 and b) [human, mRNA Partial, 284 nt]"	S76346
IKKE	IKK-related kinase epsilon; inducible IkappaB kinase	D63485
LU	Lutheran blood group (Auberger b antigen included)	X80026

KIAA0828	KIAA0828 protein	AB020635
SLC30A3	"solute carrier family 30 (zinc transporter), member 3"	U76010
IL13RA1	"interleukin 13 receptor, alpha 1"	Y10659
C22orf4	chromosome 22 open reading frame 4	AL096779
BCL11A	B-cell CLL/lymphoma 11A (zinc finger protein)	W27619
HIPK3	homeodomain interacting protein kinase 3	AI523538
ACVR1B	"activin A receptor, type IB"	Z22536
UBA2	SUMO-1 activating enzyme subunit 2	AL041443
THRA	"thyroid hormone receptor, alpha (erythroblastic leukemia viral (v-erb-a) oncogene homolog, avian)"	X55005
NCOA2	nuclear receptor coactivator 2	AI040324
IRF2	interferon regulatory factor 2	X15949
		L38424
GNAS	GNAS complex locus	X04409
TM4SF6	transmembrane 4 superfamily member 6	AF043906
ZK1	Kruppel-type zinc finger (C2H2)	AB011414
ARPC5	"actin related protein 2/3 complex, subunit 5, 16kDa"	AF006088
PEX7	peroxisomal biogenesis factor 7	U88871
FMR1	fragile X mental retardation 1	X69962
ZP2	zona pellucida glycoprotein 2 (sperm receptor)	M90366
OR7E126P	"olfactory receptor, family 7, subfamily A, member 126 pseudogene"	AF065854
HSF4	heat shock transcription factor 4	D87673
		HG2702-HT2798
UBE2G1	"ubiquitin-conjugating enzyme E2G 1 (UBC7 homolog, C. elegans)"	D78514
GRLF1	glucocorticoid receptor DNA binding factor 1	AI670100
SSFA2	sperm specific antigen 2	M61199
JIK	STE20-like kinase	W28742
PPP3CC	"protein phosphatase 3 (formerly 2B), catalytic subunit, gamma isoform (calcineurin A gamma)"	AI762547
AHCYL1	S-adenosylhomocysteine hydrolase-like 1	AI800578
PRCP	prolylcarboxypeptidase (angiotensinase C)	L13977
NR2C1	"nuclear receptor subfamily 2, group C, member 1"	M29960
FUS	"fusion, derived from t(12;16) malignant liposarcoma"	S62140
ZNF273	zinc finger protein 273	X78932
MYST1	MYST histone acetyltransferase 1	AI417075
NQO1	"NAD(P)H dehydrogenase, quinone 1"	M81600
ADAM15	a disintegrin and metalloproteinase domain 15 (metargidin)	U41767
CRYAB	"crystallin, alpha B"	AL038340
DKFZp566D133	DKFZp566D133 protein	AL050050

MAPRE1	"microtubule-associated protein, RP/EB family, member 1"	U24166
TGFB1	"transforming growth factor, beta 1 (Camurati-Engelmann disease)"	X02812
ZNF189	zinc finger protein 189	AF025770
ATP1B3	"ATPase, Na+/K+ transporting, beta 3 polypeptide"	U51478
TG737	"Probe hTg737 (polycystic kidney disease, autosomal recessive, in)"	U20362
FST	follistatin	M19481
DKFZP564O0423	DKFZP564O0423 protein	AL080120
MAGEA4	"melanoma antigen, family A, 4"	U10688
POU6F1	"POU domain, class 6, transcription factor 1"	Z21966
FLJ20986	hypothetical protein FLJ20986	Z24724
LOC90586	amine oxidase pseudogene	AF047485
MIPEP	mitochondrial intermediate peptidase	U80034
	Homo sapiens clone 24507 mRNA sequence	AF052148
	Homo sapiens mRNA; cDNA DKFZp667O1814 (from clone DKFZp667O1814)	W26677
HTR1E	5-hydroxytryptamine (serotonin) receptor 1E	M91467
DKFZP564L0862	DKFZP564L0862 protein	AL080091
HRB2	HIV-1 rev binding protein 2	U00943
REA	repressor of estrogen receptor activity	U72511
DOK1	"docking protein 1, 62kDa (downstream of tyrosine kinase 1)"	U70987
KIAA0710	KIAA0710 gene product	AB014610
PRNP	"prion protein (p27-30) (Creutzfeld-Jakob disease, Gerstmann-Strausler-Scheinker syndrome, fatal familial insomnia)"	U29185
PTK7	PTK7 protein tyrosine kinase 7	U33635
KIAA0426	KIAA0426 gene product	AB007886
	"Phosphoglycerate kinase {alternatively spliced} [human, phosphoglycerate kinase deficient patient with episodes of musc], mRNA Partial Mutant, 307 nt]"	S81916
NEDD4	"neural precursor cell expressed, developmentally down-regulated 4"	D42055
CSH2	chorionic somatomammotropin hormone 2	AA151971
ARF4	ADP-ribosylation factor 4	M36341
CD34	CD34 antigen	M81945
KIAA0092	KIAA0092 gene product	D42054
DKFZp434G2311	hypothetical protein DKFZp434G2311	W22289
GYPB	glycophorin B (includes Ss blood group)	U05255
TIC	SEC7 homolog	U63127

		X81072
KIAA0552	KIAA0552 gene product	AB011124
KIAA0970	KIAA0970 protein	AB023187
SLC18A1	"solute carrier family 18 (vesicular monoamine), member 1"	U39905
		D86096
S100A5	S100 calcium binding protein A5	Z18954
EFNA3	ephrin-A3	U14187
NM23-H6	nucleoside diphosphate kinase type 6 (inhibitor of p53-induced apoptosis-alpha)	AF051941
NXF1	nuclear RNA export factor 1	AJ132712
SLC4A8	"solute carrier family 4, sodium bicarbonate cotransporter, member 8"	AB018282
IGHM	immunoglobulin heavy constant mu	AF015128
EEF1A1	eukaryotic translation elongation factor 1 alpha 1	W28170
	Homo sapiens clone 24468 mRNA sequence	AF070623
USP9X	"ubiquitin specific protease 9, X chromosome (fat facets-like Drosophila)"	X98296
DYRK2	dual-specificity tyrosine-(Y)-phosphorylation regulated kinase 2	Y09216
LBP	lipopolysaccharide binding protein	AF013512
POH1	26S proteasome-associated pad1 homolog	U86782
KIAA0211	KIAA0211 gene product	D86966
PXR1	peroxisome receptor 1	Z48054
		HG2689-HT2785
TAF4	"TAF4 RNA polymerase II, TATA box binding protein (TBP)-associated factor, 135kDa"	U75308
ZNF313	zinc finger protein 313	AL031685
PPAP2A	phosphatidic acid phosphatase type 2A	AF014402
FLJ20323	hypothetical protein FLJ20323	AC004982
TCP1	t-complex 1	X52882
NR2F1	"nuclear receptor subfamily 2, group F, member 1"	X16155
MAG	myelin associated glycoprotein	M29273
		J04423
ELAC2	elaC homolog 2 (E. coli)	AA522537
MAPKAPK2	mitogen-activated protein kinase-activated protein kinase 2	U12779
SMAP	skeletal muscle abundant protein	X87613
ZNF263	zinc finger protein 263	D88827
DDX27	DEAD/H (Asp-Glu-Ala-Asp/His) box polypeptide 27	W25911
HSA6591	nucleolar cysteine-rich protein	AJ006591
MAGOH	"mago-nashi homolog, proliferation-associated (Drosophila)"	AF035940
		Y16788

KRT2A	keratin 2A (epidermal ichthyosis bullosa of Siemens)	AF019084
RALY	"RNA binding protein (autoantigenic, hnRNP-associated with lethal yellow)"	L38696
C11orf9	chromosome 11 open reading frame 9	AB023171
XPO1	"exportin 1 (CRM1 homolog, yeast)"	Y08614
H2BFC	"H2B histone family, member C"	AL009179
SETDB1	"SET domain, bifurcated 1"	D31891
SEC63L	SEC63 protein	AJ011779
MGC8721	hypothetical protein MGC8721	W26659
RPP40	"ribonuclease P, 40kD subunit"	U94317
GAPD	glyceraldehyde-3-phosphate dehydrogenase	M33197
KIAA0467	KIAA0467 protein	AB007936
KCNMB1	"potassium large conductance calcium-activated channel, subfamily M, beta member 1"	U25138
PML	promyelocytic leukemia	M79463
B2M	beta-2-microglobulin	S82297
UROS	uroporphyrinogen III synthase (congenital erythropoietic porphyria)	J03824
PDE4A	"phosphodiesterase 4A, cAMP-specific (phosphodiesterase E2 dunce homolog, Drosophila)"	L20965 M59830
NUP155	nucleoporin 155kDa	AB018334
HRMT1L1	HMT1 hnRNP methyltransferase-like 1 (S. cerevisiae)	X99209
BTN3A2	"butyrophilin, subfamily 3, member A2"	U97502
TRAP100	thyroid hormone receptor-associated protein (100 kDa)	W29091
PRKCD	"protein kinase C, delta"	D10495
OAZ2	ornithine decarboxylase antizyme 2	AF057297
ADRBK1	"adrenergic, beta, receptor kinase 1"	U08438
GTF2H4	"Homo sapiens cDNA FLJ30824 fis, clone FEBRA2001698" "general transcription factor IIH, polypeptide 4, 52kDa"	H12054 Y07595
LGALS9	"lectin, galactoside-binding, soluble, 9 (galectin 9)"	AB006782
ACTB	"actin, beta"	X00351
TMSB4Y	"thymosin, beta 4, Y chromosome"	AF000989
GTF3C2	"general transcription factor IIIC, polypeptide 2, beta 110kDa"	D13636
C9orf3	chromosome 9 open reading frame 3	AF043897
NSEP1	nuclease sensitive element binding protein 1	M85234
TNP1	transition protein 1 (during histone to protamine replacement)	X07948 D10995
HEXA	hexosaminidase A (alpha polypeptide)	M16424
CCNF	cyclin F	Z36714
SIP	Siah-interacting protein	AL034450 AL035305 X81832

HLA-F	"major histocompatibility complex, class I, F"	AL022723
DKFZP434D1335	DKFZP434D1335 protein	AI920820
RNASEH1	ribonuclease H1	AF039652
	"Homo sapiens cDNA: FLJ23482 fis, clone KAIA03142"	U55980
KIAA0877	KIAA0877 protein	AB020684
CLTB	"clathrin, light polypeptide (Lcb)"	X81637
HSPA8	heat shock 70kDa protein 8	Y00371
CTNNA1	"catenin (cadherin-associated protein), alpha 1 (102kDa"	U03100
		W27906
EIF4A2	"eukaryotic translation initiation factor 4A, isoform 2"	D30655
H2BFN	"H2B histone family, member N"	Z98744
KIAA0514	KIAA0514 gene product	AB011086
PRPS1	phosphoribosyl pyrophosphate synthetase 1	D00860
PAX8	paired box gene 8	X69699
		U10689
B4GALT4	"UDP-Gal:betaGlcNAc beta 1,4- galactosyltransferase, polypeptide 4"	AF038662
	Homo sapiens clone 23821 mRNA sequence	AF038194
PAFAH1B1	"platelet-activating factor acetylhydrolase, isoform Ib, alpha subunit 45kDa"	L13385
IFNA10	"interferon, alpha 10"	V00551
ABCB10	"ATP-binding cassette, sub-family B (MDR/TAP), member 10"	U18237
CASP10	"caspase 10, apoptosis-related cysteine protease"	U60519
PFKM	"phosphofructokinase, muscle"	U24183
RCN2	"reticulocalbin 2, EF-hand calcium binding domain"	X78669
PPP3CB	"protein phosphatase 3 (formerly 2B), catalytic subunit, beta isoform (calcineurin A beta)"	M29550
H6PD	hexose-6-phosphate dehydrogenase (glucose 1-dehydrogenase)	AJ012590
PTPRA	"protein tyrosine phosphatase, receptor type, A"	M34668
FUT7	"fucosyltransferase 7 (alpha (1,3) fucosyltransferase)"	AB012668
PFKP	"phosphofructokinase, platelet"	D25328
MAGEA9	"melanoma antigen, family A, 9"	U10694
SDFR1	stromal cell derived factor receptor 1	AF035287
CAV2	caveolin 2	AF035752
ERCC5	"excision repair cross-complementing rodent repair deficiency, complementation group 5 (xeroderma pigmentosum, complementation group G (Cockayne syndrome))"	L20046
MLN	motilin	X15393
PTK2	PTK2 protein tyrosine kinase 2	L13616
P84	nuclear matrix protein p84	L36529
OS4	conserved gene amplified in osteosarcoma	AF000152

ITPR2	"inositol 1,4,5-triphosphate receptor, type 2"	D26350
POU6F1	"POU domain, class 6, transcription factor 1"	Z21966
GATA2	GATA binding protein 2	M77810
SFRS7	"splicing factor, arginine/serine-rich 7, 35kDa"	L41887
FBXO21	F-box only protein 21	AB020682
AGM1	N-acetylglucosamine-phosphate mutase	AA001791
UGT2B15	"UDP glycosyltransferase 2 family, polypeptide B15"	U06641
SGNE1	"secretory granule, neuroendocrine protein 1 (7B2 protein)"	Y00757
CHP	calcium binding protein P22	U61538
PDCD10	programmed cell death 10	AF022385
FLJ21432	hypothetical protein FLJ21432	W26655
KIAA0692	KIAA0692 protein	AI924382
HNRPH3	heterogeneous nuclear ribonucleoprotein H3 (2H9)	AF052131
OCRL	oculocerebrorenal syndrome of Lowe	U57627
ESR2	estrogen receptor 2 (ER beta)	X99101
		HG1111-HT1111
	Homo sapiens mRNA; cDNA DKFZp586I1319 (from clone DKFZp586I1319)	AL050106
SIM2	single-minded homolog 2 (Drosophila)	U80457
DCTN1	"dynactin 1 (p150, glued homolog, Drosophila)"	AF086947
MGC9651	hypothetical protein MGC9651	W21884
SFRS3	"splicing factor, arginine/serine-rich 3"	AF038250
ZNF10	zinc finger protein 10 (KOX 1)	X52332
AP2A2	"adaptor-related protein complex 2, alpha 2 subunit"	AB020706
FLJ10618	hypothetical protein FLJ10618	AL049246
TTTY15	"testis-specific transcript, Y-linked 15"	AL080135
ID1	"inhibitor of DNA binding 1, dominant negative helix-loop-helix protein"	X77956
DAG1	dystroglycan 1 (dystrophin-associated glycoprotein 1)	L19711
ZNF175	zinc finger protein 175	D50419
		W26472
RAB2	"RAB2, member RAS oncogene family"	M28213
ENPP4	ectonucleotide pyrophosphatase/phosphodiesterase 4 (putative function)	AB020686
RHBDL	"rhomboid, veinlet-like 1 (Drosophila)"	Y17108
KIAA0648	KIAA0648 protein	AB014548
UCHL3	ubiquitin carboxyl-terminal esterase L3 (ubiquitin thiolesterase)	AA746355
LOC51035	ORF	M68864
ITGB2	"integrin, beta 2 (antigen CD18 (p95), lymphocyte function-associated antigen 1; macrophage antigen 1 (mac-1) beta subunit)"	M15395
PPP2R5C	"protein phosphatase 2, regulatory subunit B (B56), gamma isoform"	Z69030

MIR16	membrane interacting protein of RGS16	AC003108
HSPCB	"heat shock 90kDa protein 1, beta"	M16660
ATP6V1A1	"ATPase, H+ transporting, lysosomal 70kDa, V1 subunit A, isoform 1"	AA056747
CETN3	"centrin, EF-hand protein, 3 (CDC31 homolog, yeast)"	AI056696
PRDX3	peroxiredoxin 3	D49396
LOC129080	putative emu1	AL031186
P2RX5	"purinergic receptor P2X, ligand-gated ion channel, 5"	U49395
HUMPPA	paraneoplastic antigen	L02867
		HG2530-HT2626
SCAP	SREBP CLEAVAGE-ACTIVATING PROTEIN	D83782
MD-1	"MD-1, RP105-associated"	AB020499
CDC6	CDC6 cell division cycle 6 homolog (S. cerevisiae)	U77949
BRAP	BRCA1 associated protein	AL042733
CAMK2G	calcium/calmodulin-dependent protein kinase (CaM kinase) II gamma	U66063
MLCB	"myosin, light polypeptide, regulatory, non-sarcomeric (20kD)"	X54304
OPA1	optic atrophy 1 (autosomal dominant)	AB011139
HSPC111	hypothetical protein HSPC111	AI553745
STK39	"serine threonine kinase 39 (STE20/SPS1 homolog, yeast)"	AF099989
YME1L1	YME1-like 1 (S. cerevisiae)	AJ132637
H1F2	"H1 histone family, member 2"	AI189287
MLANA	melan-A	U06452
PSMD9	"proteasome (prosome, macropain) 26S subunit, non-ATPase, 9"	AI347155
LARGE	like-glycosyltransferase	AJ007583
CREB3	cAMP responsive element binding protein 3 (luman)	U88528
MRPS14	mitochondrial ribosomal protein S14	AL049705
TM4SF5	transmembrane 4 superfamily member 5	AF027204
SIT	SHP2 interacting transmembrane adaptor	AJ010059
EPB49	erythrocyte membrane protein band 4.9 (dematin)	Z48950 U28389
TCN2	transcobalamin II; macrocytic anemia	L02648
OIP2	Opa-interacting protein 2	AL050353
ALAS2	"aminolevulinate, delta-, synthase 2 (sideroblastic/hypochromic anemia)"	X60364
CHC1	chromosome condensation 1	X12654
GMPS	guanine monphosphate synthetase	U10860
SLC25A14	"solute carrier family 25 (mitochondrial carrier, brain), member 14"	AF078544
HNRPM	heterogeneous nuclear ribonucleoprotein M	L03532
PDZ-GEF1	PDZ domain containing guanine nucleotide exchange factor(GEF)1	AB002311
UBE2N	"ubiquitin-conjugating enzyme E2N (UBC13 homolog,	D83004

	yeast)"	
	"ESTs, Moderately similar to hypothetical protein FLJ20489 [Homo sapiens] [H.sapiens]"	W28230
NEDD5	"neural precursor cell expressed, developmentally down-regulated 5"	M11717 D63878 J04423
CDH2	"cadherin 2, type 1, N-cadherin (neuronal)"	M34064
PP35	protein similar to E.coli yhdg and R. capsulatus nifR3	U62767
	Homo sapiens mRNA; cDNA DKFZp686N1377 (from clone DKFZp686N1377)	S63912
	"Homo sapiens cDNA FLJ13555 fis, clone PLACE1007677"	AL080210 M33764
RELN	reelin	U79716
PPP1R12A	"protein phosphatase 1, regulatory (inhibitor) subunit 12A"	D87930
SLC9A6	"solute carrier family 9 (sodium/hydrogen exchanger), isoform 6"	AF030409
NRXN1	neurexin 1	AB011150
76P	gamma tubulin ring complex protein (76p gene)	W28255
DKFZp564B0769	SR rich protein	AL080186
ADPRT	ADP-ribosyltransferase (NAD+; poly (ADP-ribose) polymerase)	J03473
SRPX	"sushi-repeat-containing protein, X chromosome"	U61374
SAS10	disrupter of silencing 10	AI126004
GNAS	GNAS complex locus	X04409 X57152
MID2	midline 2	AL034399
U5-100K	"prp28, U5 snRNP 100 kd protein"	AF026402
PTPRD	"protein tyrosine phosphatase, receptor type, D"	AA843737
SPTB	"spectrin, beta, erythrocytic (includes spherocytosis, clinical type I)"	J05500
CDK6	cyclin-dependent kinase 6	AI738463
DPYSL4	dihydropyrimidinase-like 4	AB006713
DKFZP566F0546	DKFZP566F0546 protein	AI671905
CCT2	"chaperonin containing TCP1, subunit 2 (beta)"	AF026166
PROL2	proline rich 2	U03105
		D00591
		M13929
DR1	"down-regulator of transcription 1, TBP-binding (negative cofactor 2)"	M97388
MTHFR	"5,10-methylenetetrahydrofolate reductase (NADPH)"	L00049 AJ237672
SIMRP7	multidrug resistance-associated protein 7	AI004207

CDH11	"cadherin 11, type 2, OB-cadherin (osteoblast)"	D21255
FLJ11198	hypothetical protein FLJ11198	U66685
ATRX	"alpha thalassemia/mental retardation syndrome X-linked (RAD54 homolog, S. cerevisiae)"	U72936
BRCA1	"breast cancer 1, early onset"	U64805
MLLT4	"myeloid/lymphoid or mixed-lineage leukemia (trithorax homolog, Drosophila); translocated to, 4"	AB011399
COX11	"COX11 homolog, cytochrome c oxidase assembly protein (yeast)"	U79270
TCEA1	"transcription elongation factor A (SII), 1"	M81601
TEGT	testis enhanced gene transcript (BAX inhibitor 1)	X75881
RPL9	ribosomal protein L9	U09953
CDK5R1	"cyclin-dependent kinase 5, regulatory subunit 1 (p35)"	X80343
		HG4518-HT4921
SOS2	son of sevenless homolog 2 (Drosophila)	L13858
EPHB2	EphB2	AF025304
		Z97054
KIAA0185	KIAA0185 protein	D80007
MYC	v-myc myelocytomatosis viral oncogene homolog (avian)	V00568
KCNK3	"potassium channel, subfamily K, member 3"	AF006823
HSPA9B	heat shock 70kDa protein 9B (mortalin-2)	L15189
AIF1	allograft inflammatory factor 1	Y14768
PMS2L6	postmeiotic segregation increased 2-like 6	AI341574
DMWD	dystrophia myotonica-containing WD repeat motif	L19267
GMPR	guanosine monophosphate reductase	M24470
RTP801	HIF-1 responsive RTP801	M10098 AA522530
MMP11	matrix metalloproteinase 11 (stromelysin 3)	X57766
KIAA1067	KIAA1067 protein	AB028990
ADAM19	a disintegrin and metalloproteinase domain 19 (meltrin beta)	AL049415
	Homo sapiens mRNA; cDNA DKFZp586F2224 (from clone DKFZp586F2224)	AI655015
C1orf16	chromosome 1 open reading frame 16	D87437
GP1BA	"glycoprotein Ib (platelet), alpha polypeptide"	J02940
SDHB	"succinate dehydrogenase complex, subunit B, iron sulfur (Ip)"	U17886
NTRK2	"neurotrophic tyrosine kinase, receptor, type 2"	U12140
KIAA0110	gene predicted from cDNA with a complete coding sequence	D14811
MAP3K7	mitogen-activated protein kinase kinase kinase 7	AB009356
MGC5466	hypothetical protein MGC5466	U90904
PPM1A	"protein phosphatase 1A (formerly 2C), magnesium-dependent, alpha isoform"	S87759
		K01383

KIAA0677	KIAA0677 gene product	AB014577
HNRPA2B1	heterogeneous nuclear ribonucleoprotein A2/B1	M29065
DKFZP434J046	DKFZP434J046 protein	AC004144
MAN1A1	"mannosidase, alpha, class 1A, member 1"	X74837
KIAA0455	KIAA0455 gene product	AB007924
NUP160	nucleoporin 160kDa	D83781
NMT1	N-myristoyltransferase 1	M86707
PIP5K1C	"phosphatidylinositol-4-phosphate 5-kinase, type I, gamma"	AB011161
GTF2H3	"general transcription factor IIH, polypeptide 3, 34kDa"	Z30093
DCN	decorin	M14219
	"Human small proline rich protein (sprII) mRNA, clone 174N"	M21302
POLR2B	"polymerase (RNA) II (DNA directed) polypeptide B, 140kDa"	X63563
AHSG	alpha-2-HS-glycoprotein	J04988 M16961
STAM	signal transducing adaptor molecule (SH3 domain and ITAM motif) 1	U43899
SCAM-1	vinexin beta (SH3-containing adaptor molecule-1)	AF037261
RAF1	v-raf-1 murine leukemia viral oncogene homolog 1	X06409
KIAA0964	KIAA0964 protein	AB023181
SPARCL1	"SPARC-like 1 (mast9, hevin)"	X86693
PGRMC1	progesterone receptor membrane component 1	Y12711
COPS5	COP9 constitutive photomorphogenic homolog subunit 5 (Arabidopsis)	U65928
MGC2650	hypothetical protein MGC2650	AI885381
CYP11A	"cytochrome P450, subfamily XIA (cholesterol side chain cleavage)"	M14565
CPB2	"carboxypeptidase B2 (plasma, carboxypeptidase U)"	M75106
NRG1	neuregulin 1	L41827
GTF2F2	"general transcription factor IIF, polypeptide 2, 30kDa"	X16901
UCP2	"uncoupling protein 2 (mitochondrial, proton carrier)"	U94592
BM036	uncharacterized bone marrow protein BM036	AI057607
HLA-G	"HLA-G histocompatibility antigen, class I, G"	M90683
SS18L1	synovial sarcoma translocation gene on chromosome 18-like 1	AB014593
DKFZP547E1010	DKFZP547E1010 protein	AL050260
PARG	poly (ADP-ribose) glycohydrolase	AF005043
RPS15A	ribosomal protein S15a	W52024
CREBL2	cAMP responsive element binding protein-like 2	AF039081
HSD17B3	hydroxysteroid (17-beta) dehydrogenase 3	U05659
	Homo sapiens clone 23718 mRNA sequence	AF052138
		HG2465-HT4871

IDI1	isopentenyl-diphosphate delta isomerase	X17025
CBX3	"chromobox homolog 3 (HP1 gamma homolog, Drosophila)"	AA648295
PAI-RBP1	PAI-1 mRNA-binding protein	AL080119
SFPQ	splicing factor proline/glutamine rich (polypyrimidine tract binding protein associated)	W27050
AMACR	alpha-methylacyl-CoA racemase	AJ130733
KIAA1045	KIAA1045 protein	AB028968
HNRPH2	heterogeneous nuclear ribonucleoprotein H2 (H')	U01923
KIAA0537	KIAA0537 gene product	AB011109
		X55503
MLLT2	"myeloid/lymphoid or mixed-lineage leukemia (trithorax homolog, Drosophila); translocated to, 2"	L13773
ELAVL3	"ELAV (embryonic lethal, abnormal vision, Drosophila)-like 3 (Hu antigen C)"	D28158
ING1L	"inhibitor of growth family, member 1-like"	AI186701
PPP4R1	"protein phosphatase 4, regulatory subunit 1"	U79267
ACTB	"actin, beta"	X63432
FBXO9	F-box only protein 9	AL031178
LYPLA1	lysophospholipase I	AF081281
POLR3F	"polymerase (RNA) III (DNA directed) polypeptide F, 39 kDa"	U93869
MCLC	Mid-1-related chloride channel 1	AB018304
PPIE	peptidylprolyl isomerase E (cyclophilin E)	AF042386
PAICS	"phosphoribosylaminoimidazole carboxylase, phosphoribosylaminoimidazole succinocarboxamide synthetase"	X53793
IFNGR2	interferon gamma receptor 2 (interferon gamma transducer 1)	U05875
PITPNM	"phosphatidylinositol transfer protein, membrane-associated"	X98654
		X03453
KIAA0435	KIAA0435 gene product	AB007895
TAZ	"tafazzin (cardiomyopathy, dilated 3A (X-linked); endocardial fibroelastosis 2; Barth syndrome)"	X92762
ATP6V1H	"ATPase, H+ transporting, lysosomal 50/57kDa, V1 subunit H"	AI741756
DKFZP566C243	DKFZP566C243 protein	AL050274
PPP1R3D	"protein phosphatase 1, regulatory subunit 3D"	Y18206
SBA2	CS box-containing WD protein	AF038187
MEF2A	"MADS box transcription enhancer factor 2, polypeptide A (myocyte enhancer factor 2A)"	U49020 J05614
UNC13	unc-13-like (C. elegans)	AF020202
HFL-EDDG1	erythroid differentiation and denucleation factor 1	AF048849
LTA4H	leukotriene A4 hydrolase	J03459

METTL1	methyltransferase-like 1	Y18643 AD000092
	"Homo sapiens cDNA FLJ40021 fis, clone STOMA2006904"	AL080094
IFIT1	interferon-induced protein with tetratricopeptide repeats 1	M24594
TEF	thyrotrophic embryonic factor	U44059
HMOX2	heme oxygenase (decycling) 2	AI086057
DDB1	"damage-specific DNA binding protein 1, 127kDa"	U32986
AKAP8	A kinase (PRKA) anchor protein 8	Y11997
SLC9A1	"solute carrier family 9 (sodium/hydrogen exchanger), isoform 1 (antiporter, Na+/H+, amiloride sensitive)"	S68616
ACADM	"acyl-Coenzyme A dehydrogenase, C-4 to C-12 straight chain"	M91432
NEURL	neurallzed-like (Drosophila)	AF029729
CDKN1B	"cyclin-dependent kinase inhibitor 1B (p27, Kip1)"	AI304854
ASH2L	"ash2 (absent, small, or homeotic)-like (Drosophila)"	AB022785
KHDRBS1	"KH domain containing, RNA binding, signal transduction associated 1"	M88108
SNAP25	"synaptosomal-associated protein, 25kDa"	D21267
RP2	retinitis pigmentosa 2 (X-linked recessive)	AJ007590
ACAT2	acetyl-Coenzyme A acetyltransferase 2 (acetoacetyl Coenzyme A thiolase)	S70154
ATP6V1A1	"ATPase, H+ transporting, lysosomal 70kDa, V1 subunit A, isoform 1"	L09235
AQP1	"aquaporin 1 (channel-forming integral protein, 28kDa)"	U41518
PPP1R8	"protein phosphatase 1, regulatory (inhibitor) subunit 8"	U14575
HLA-DOB	"major histocompatibility complex, class II, DO beta"	X03066
ENSA	endosulfine alpha	X99906
MXI1	MAX interacting protein 1	L07648
PSMD4	"proteasome (prosome, macropain) 26S subunit, non-ATPase, 4"	U51007
SLC6A2	"solute carrier family 6 (neurotransmitter transporter, noradrenalin), member 2"	X91117
GTF2I	"general transcription factor II, I"	U77948
		M35093
ZFP36L2	"zinc finger protein 36, C3H type-like 2"	U07802
NUP98	nucleoporin 98kDa	AF042357
MYOZ3	myozenin 3	AF052497
NF1	"neurofibromin 1 (neurofibromatosis, von Recklinghausen disease, Watson disease)"	D12625
	Homo sapiens mRNA; cDNA DKFZp564O0122 (from clone DKFZp564O0122)	AL049951
PSMC2	"proteasome (prosome, macropain) 26S subunit, ATPase, 2"	D11094
PPP3CB	"protein phosphatase 3 (formerly 2B), catalytic subunit, beta isoform (calcineurin A beta)"	M29551

ITGA2B	"integrin, alpha 2b (platelet glycoprotein IIb of IIb/IIIa complex, antigen CD41B)"	M34480
FGF18	fibroblast growth factor 18	AF075292
PYCR1	pyrroline-5-carboxylate reductase 1	M77836
EIF4B	eukaryotic translation initiation factor 4B	X55733
KIAA0806	KIAA0806 gene product	R93981
	"Homo sapiens cDNA FLJ31348 fis, clone MESAN2000026"	AI970189
		AC002073
MGC5576	hypothetical protein MGC5576	W27939
UBE2E1	"ubiquitin-conjugating enzyme E2E 1 (UBC4/5 homolog, yeast)"	AI039880
JJAZ1	joined to JAZF1	D63881
PMS1	PMS1 postmeiotic segregation increased 1 (S. cerevisiae)	U13695
KIAA0240	KIAA0240 protein	D87077
TBCD	tubulin-specific chaperone d	AJ006417
NUP214	nucleoporin 214kDa	X64228
FOSL2	FOS-like antigen 2	X16706
PAFAH1B1	"platelet-activating factor acetylhydrolase, isoform Ib, alpha subunit 45kDa"	L25107
PSMA1	"proteasome (prosome, macropain) subunit, alpha type, 1"	M64992
	ESTs	AI184710
APOBEC3B	"apolipoprotein B mRNA editing enzyme, catalytic polypeptide-like 3B"	AL022318
		U18671
H41	hypothetical protein H41	H15872
		HG4582-HT4987
ORC1L	"origin recognition complex, subunit 1-like (yeast)"	U40152
XDH	xanthene dehydrogenase	U39487
	Homo sapiens mRNA; cDNA DKFZp434M162 (from clone DKFZp434M162)	W72239
FUBP3	far upstream element (FUSE) binding protein 3	U69127
ID1	"inhibitor of DNA binding 1, dominant negative helix-loop-helix protein"	S78825
KIAA0637	KIAA0637 gene product	AB014537
CLTB	"clathrin, light polypeptide (Lcb)"	M20470
KIAA1094	KIAA1094 protein	AB029017
RAB1A	"RAB1A, member RAS oncogene family"	M28209
ERCC6	"excision repair cross-complementing rodent repair deficiency, complementation group 6"	L04791
MYT1	myelin transcription factor 1	AB028973
MGC10471	hypothetical protein MGC10471	X13956
C12orf8	chromosome 12 open reading frame 8	X94910

MSL3L1	male-specific lethal 3-like 1 (Drosophila)	AL050178
CSTF2T	likely ortholog of mouse variant polyadenylation protein CSTF-64	AB014589
GS3955	GS3955 protein	D87119
		U14573
MTA1	metastasis associated 1	U35113
FLJ20619	hypothetical protein FLJ20619	AL049431
DNAJC7	"DnaJ (Hsp40) homolog, subfamily C, member 7"	W28595
TFRC	"transferrin receptor (p90, CD71)"	X01060
KIAA0218	KIAA0218 gene product	D86972
KIAA1089	KIAA1089 protein	AB029012
FCGR2A	"Fc fragment of IgG, low affinity IIa, receptor for (CD32)"	M31932
CSNK1A1	"casein kinase 1, alpha 1"	L37042
HPS1	Hermansky-Pudlak syndrome 1	U65676
ACK1	activated p21cdc42Hs kinase	L13738
MAP-1	modulator of apoptosis 1	AI670788
DDX9	"DEAD/H (Asp-Glu-Ala-Asp/His) box polypeptide 9 (RNA helicase A, nuclear DNA helicase II; leukophysin)"	L13848
FAM8A1	"family with sequence similarity 8, member A1"	AL050128
PRO2730	hypothetical protein PRO2730	AL045811
	Homo saplens mRNA; cDNA DKFZp586H201 (from clone DKFZp586H201)	AL049430
KIAA0146	KIAA0146 protein	D63480
NUDEL	LIS1-interacting protein NUDEL; endooligopeptidase A	AF038203
ARC	activity-regulated cytoskeleton-associated protein	D87468
HMBS	hydroxymethylbilane synthase	M95623
TRA1	tumor rejection antigen (gp96) 1	X15187
		U12471
DAP	death-associated protein	X76105
RYBP	RING1 and YY1 binding protein	AL049940
RGS19	regulator of G-protein signalling 19	X91809
BMP10	bone morphogenetic protein 10	AF101441
KIAA0492	KIAA0492 protein	AB007961
URKL1	uridine kinase-like 1	AI249721
SFRS2	"splicing factor, arginine/serine-rich 2"	X75755
CAPNS1	"calpain, small subunit 1"	X04106
C1orf8	chromosome 1 open reading frame 8	Z78368
UBE3B	ubiquitin protein ligase	AI749193
E2F3	E2F transcription factor 3	D38550
		J04423
USP1	ubiquitin specific protease 1	AB014458
TNRC15	trinucleotide repeat containing 15	AB014542
IL5RA	"interleukin 5 receptor, alpha"	M75914
		X03453
RHEB2	Ras homolog enriched in brain 2	D78132

LSM6	Sm protein F	AA917945
TBX5	T-box 5	Y09445
	Homo sapiens mRNA; cDNA DKFZp451N147 (from clone DKFZp451N147)	AA534868
ARSE	arylsulfatase E (chondrodysplasia punctata 1)	X83573
LCP1	lymphocyte cytosolic protein 1 (L-plastin)	J02923
CSF1	colony stimulating factor 1 (macrophage)	M37435
DHCR7	7-dehydrocholesterol reductase	AF034544

Recent technical developments have now facilitated the analysis of large numbers of genes by means of the use of high density microarrays or "chips". Each location on such a chip contains a sequence related to a specific sequence, such that when a signal (such as a visual color, produced by the use of suitable colored conjugate) is present, it can be readily related to the binding of sequences specific for a particular gene, the identity of which is determined by the position of the signal in the array. Suitable computer programs may then be used to analyze and present (in graphical and/or tabular form) the data extracted from the microarray signals. In addition to providing information relating to the expression of specific genes, high density microarrays may also be used to generate "fingerprints" which are characteristic of, for example, a particular disease, treatment response or (as in the case of the invention disclosed herein) prognostic group. The fingerprint thus obtained may be subjected to analysis by any of a number of statistical techniques (including

cluster analysis, as described in the illustrative example, hereinbelow), in order to assign said fingerprint to a discrete results group. The results group may be one of a binary pair (such as the good prognosis/poor prognosis pair of the present invention), or it may be one of a more complex series of groups (such as in the case of the differential diagnosis of several pathological possibilities.)

Suitable high density microarrays may either be purchased "off-the-shelf", pre-loaded with an array of oligonucleotide sequences (for example the Genechip Human Genome arrays produced by Affymetrix, Santa Clara, CA, USA), or alternatively may be custom-produced such that they bear a subset of the total genome, wherein said subset is relevant for the desired diagnostic, prognostic or drug discovery application of the microarray. Many different materials and techniques may be used in the construction of high density microarrays, the details of which appear in many publications including US 6,344,316, which is in its entirety incorporated herein by reference.

The techniques used to obtain, purify and hybridize RNA and other nucleic acids are varied and well known to all skilled artisans in the field. Details of many such suitable techniques are to be found in standard reference works such as the book "Molecular cloning: a laboratory manual" by Sambrook, J., Fritsch, E.F. & Maniatis, T., Cold Spring Harbor, NY, 2nd ed., 1989 (and all later editions), which is incorporated herein by reference in its entirety.

In addition, Methods of isolating total mRNA are described in detail in Chapter 3 of Laboratory Techniques in Biochemistry and Molecular Biology: Hybridization with Nucleic Acid Probes, Part I. Theory and Nucleic Acid Preparation, P. Tijssen, ed. Elsevier, N.Y. (1993). More specific information related to the use of polymerase chain reaction (PCR) techniques may be gleaned from "Innis *et al.* eds., *PCR Protocols: A guide to method and applications*", which is incorporated herein by reference.

Following isolation of the nucleic acids sequences and their purification and hybridization to a suitable high density chip, binding is determined by means of a suitable detection method. In a preferred embodiment, the hybridized nucleic acids are detected by detecting one or more labels attached to the sample nucleic acids. The labels may be incorporated by any of a number of means well known to those of skill in the art. Labels may be introduced either during the course of the synthesis of the nucleic acid sequences (e.g. during a PCR reaction) or as a discrete post-synthetic step. Detectable labels suitable for use in the present invention include any composition detectable by spectroscopic, photochemical, biochemical, immunochemical, electrical, optical or chemical means. Particularly preferred are labels such as biotin for staining with labeled streptavidin conjugate, magnetic beads (e.g., Dynabeads.TM.), fluorescent dyes (e.g., fluorescein, texas red, rhodamine, green fluorescent protein, and the like (obtainable from Molecular Probes,

Eugene, Oregon, USA). However, other label types, including radiolabels and enzymes may also be usefully employed.

Several different types of microarray may be used or produced in order to work the present invention. Thus, a variety of different substrate types, including (but not limited to) metal oxides, nylon, ceramic material and glasses may be used to construct the microarray. In a commonly-used configuration, the microarray is constructed such it has a surface area less than 6.25 cm^2, preferably in the range of about 1.6 cm^2 to 6.25 cm^2. Details of the construction of microarrays suitable for use in the present invention are now well known in the art, and may be obtained from a variety of publications including the aforementioned US 6,344,316, US 6,232,068 and US 5,510,270, all of which are incorporated herein in their entirety.

The following example is provided for illustrative purposes and in order to more particularly explain and describe the present invention. The present invention, however, is not limited to the particular embodiments disclosed in the example.

Example 1

Prognosis determination by means of genetic profiling of tumor material obtained from ES patients

Methods

Patient samples

Fourteen primary tumor specimens and six metastases were obtained from 18 ES patients with non-metastatic disease. In the case of one patient, both primary and recurrent tumors were analyzed (SA37 and SA43), and two metastases were taken from another patient, six years apart (SA45 and SA46). All patients were admitted to the Pediatric Hematology Oncology Department at Schneider Children's Medical Center, Petach Tikva, Israel. Informed consent was obtained from the patients or their guardians, and the local and National Ethics Committees approved the research project. All patients were treated with a combination of aggressive chemotherapy, radiotherapy and surgery. Median age at diagnosis was 15 years (range 7-27). Five patients were females and 13 were males. Response to therapy was defined by histopathological response and assessed by percentage of tumor necrosis at the time of surgery (limb salvage procedure) following neoadjuvant chemotherapy and radiotherapy. Median follow up was 72.5 months (range 7-171). Tumors were snap-frozen in liquid nitrogen immediately after surgery and stored at -80 °C until use.

Microarray hybridization

Ten µg of total RNA was extracted from each tumor using Tri Reagent (Molecular Research Center, Inc. Cincinnati, Ohio). Double stranded cDNA was generated from 10ug of total RNA using the SuperScript Choice System from Gibco Brl (Rockville, MD, USA), using an oligo$(dT)_{24}$ primer containing a T7 promoter site at the 3' end (Genset, La Jolla, CA). cDNAs were purified via a phenol-chloroform extraction followed by an ethanol precipitation. Purified cDNA was used as template for *In vitro* transcription (IVT), which was performed with T7 RNA polymerase and biotin-labeled ribonucleotides, using the ENZO BioArray High Yield RNA Transcript Labeling Kit (Enzo Diagnostics, New York, NY). Labeled *in vitro* transcripts were purified over RNeasy mini columns (Qiagen, Valencia, CA) according to manufacturer's instructions. The labeled cRNA was fragmented at 94°C for 35 min in fragmentation buffer (40 mM Tris-acetate, pH 8.1/100 mM potassium acetate, 30 mM magnesium acetate), and a hybridization mix was generated by addition of herring sperm DNA (0.1 mg/ml), acetylated BSA (0.5 mg/ml, Invitrogen), sodium chloride (1 M), Tris-acetate (10 mM), and Tween-20 (0.0001%). A mixture of four control bacterial and phage cRNA (1.5 pM BioB, 5 pM BioC, 25 pM BioD, and 100 pM Cre) was included to serve as an internal control for hybridization efficiency.

Aliquots of each sample (12 µg cRNA in 200 µl hybridization mix) were hybridized to a Genechip Human Genome U95Av2 array (Affymetrix, Santa Clara, CA, USA). After

hybridization, each array was washed according to procedures developed by the manufacturer (Affymetrix), and stained with streptavidin-phycoerythrin conjugate (Molecular Probes, Eugene, OR). The hybridization signal was amplified by using biotinylated anti-streptavidin antibodies (Vector Laboratories, Burlingame, CA), followed by restaining with streptavidin phycoerythrin. Arrays were scanned by the GeneArray scanner G2500A (Hewlett Packard, Palo Alto, CA), and scanned images were visually inspected for hybridization imperfections. Arrays were analyzed using Genechip 4.1 software (Affymetrix). The expression value for each gene was determined by calculating the average differences of the probe pairs in use for that gene.
Two samples were analyzed in duplicate and results were reproducible.

Data analysis:

Normalization and filtering

The microarray results were analyzed using the GeneSpring Software®. Normalization was performed by setting expression values lower than zero to zero and than each measurement was divided by the median of all measurements in that sample.

In order to filter out genes that are not expressed in any of the groups, Affymetrix absolute call (MAS 4.0: P, M - expressed genes, A - not expressed) was used. Genes that were expressed in one group were defined as genes expressed in at least 3 samples.

Selecting for differentially expressed genes

A Student's t-test was applied for each gene, and genes with an adjusted *P*-value less then 0.01 were selected as differentially expressed genes. *P*-values were corrected to reduce false positive using Benjamini and Hochberg False Discovery Rate [Benjamini, Y. et al. J. Roy. Stat. Soc. B., 57, 289-300 (1995)].

Hierarchical clustering

Divisive hierarchical clustering [Everitt, B.S. Cluster analysis. 3rd edition, 62-65 (Arnold, London, 1993)] was performed as described by Eiesen et al. [Eisen, M.B. et al. *Proc. Natl. Acad. Sci. USA* 95, 14863-14868 (1998], using centered correlation as the measurement distance.

Progression free survival analysis

Kaplan-Meier progression free survival analysis, using the log rank test, was performed in order to correlate the microarray classification results with patients' clinical outcome.

Quantitative Real Time PCR (RQ-PCR)

The microarray derived expression data was evaluated for the cadherin-11 and MTA1 genes using quantitative PCR by the LightCycler system (Roche Diagnostics, Manheim, Germany). cDNA was prepared using the Reverse Transcription System (Promega Corporation, Madison, Wisconsin) and

purified with GFX PCR DNA and Gel Band Purification kit (Amersham Biosciences, Piscataway, New Jersey). 5 µl was amplified in a 20 µl reaction containing 4 mM $MgCl_2$, 5 µM of each primer and LightCycler - FastStart DNA Master SYBR Green I mix (Roche Diagnostics).
Cadherin-11 primers: sense 5'-AGAGGCCTACATTCTGAACG-3' and antisense 5'-TTCTTTCTTTTGCCTTCTCAGG-3'. MTA1 primers: sense 5'-AGCTACGAGCAGCACAACGGGGT-3' and antisense 5'-CACGCTTGGTTTCCGAGGAT-3'.
All examinations were performed in duplicate and data analysis was done using the LightCycler Software.

Results

The study included 14 tumor samples from localized ES patients. The gene expression profile of 7 tumors from patients who had progressed between 5 months up to 5 years from diagnosis (defined as High Risk - HR) was compared with 7 tumors from patients who were disease free for a long period of follow up (median 92 months; range 66-171) (defined as Low Risk - LR).

In brief, RNA was isolated from each tumor and hybridized to Affymetrix oligonucleotide high-density arrays U95Av2. A subset of genes that distinguish between the two groups (HR and LR) by two steps was identified. Firstly, 8098 genes that were expressed in one of the groups, in at least 3 samples, were selected. Subsequently, 818 genes differentially expressed in either the HR or the LR groups

(t-test; $P<0.01$) were studied. These 818 most significant genes are listed in Table 1, hereinabove.

In order to control false positive results as a consequence of multiple comparisons, the *P* values were adjusted using the False Discovery Rate (FDR) method [Everitt, B.S. Cluster analysis. 3rd edition, 62-65 (Arnold, London, Benjamin, Y. *et al.*, J. Roy. Stat. Soc. B, 57, 289 - 300 (1995)].

Using hierarchical clustering, based on the 818 genes, for prognosis profile, two distinct clusters could be determined: poor and good prognosis signatures (Fig. 1*a*). All of the seven HR and six out of the seven LR patients (86%) were classified as poor and good prognosis signatures, respectively (Table 2). One clinically LR patient who was disease free for a long period of follow up (97 months), was classified in the poor prognosis signature group. Each one of the 818 genes is sufficient for the prediction of prognosis.

Table 2: Clinical data, disease course and results of molecular classification

Sample	Age (years)	Primary Site	Response to therapy % necrosis	Relapse (months)	Outcome (months)	Microarray classification prognosis group
High Risk						
SA3	21	Pelvis	<90%	Local (5)	EX (7)	Poor
SA37	7	Cranium	N.D	Local (29)	EX (44)	Poor
SA38	17	Pelvis	<90%	Local (10)	EX (18)	Poor
SA47	20	Pelvis	>90%	Cranium (61)	AWD (76)	Poor
SA75	18	Pelvis	<90%	Local (27)	EX (49)	Poor
SA78	24	Femur	<90%	Lung (47)	EX (65)	Poor
SA79	12	Pelvis	>90%	Bone (41)	EX (60)	Poor
Low Risk						
SA2	15	Pelvis	>90%	-	NED (103)	Poor
SA4	14	Chest	N.D	-	NED (92)	Good
SA5	13	Radius	<90%	-	NED (66)	Good
SA9	13	Tibia		>90%	NED (168)	Good
SA80	15	Pelvis	>90%	-	NED (81)	Good
SA81	14	Pelvis	>90%	-	NED (82)	Good
SA82	11	Tibia		>90%	NED (173)	Good
Metastases						
SA43	7	Cranium	N.D	Local (29)	EX (44)	Poor
SA44	27	Femur	>90%	Lung (61)	NED (91)	Good
SA45	16	Femur	<90%	Brain (128)	AWD (151)	Poor
SA46	16	Femur	<90%	Lung (67)	AWD (151)	Poor
SA76	20	Pelvis	<90%	Lung (24)	EX (44)	Poor
SA77	8	Pelvis	<90%	Local (37)	EX (104)	Good

EX=Expired; NED=No Evidence of Disease; AWD=Alive With Disease
Numbers in brackets=time from diagnosis; N.D=not done

Kaplan-Meier life table analysis indicated that the patients predicted to have a good prognosis signature had a significantly improved progression free survival (PFS) compared with those predicted to have a poor prognosis signature (Fig. 1b, P=0.002).

Additionally, the genes were reordered into 2 major clusters that were divided into 6 sub-clusters, by performing hierarchical clustering of all signature genes (Fig. 1*c*). The two major groups correspond to (i) over-expressed in the poor prognosis group and down-regulated in the good prognosis group, and (ii) vice versa. The six sub-clusters correspond to the variability of genes among the patients with poor or good prognosis signatures, which was more considerable in the poor prognosis group. Genes that were over-expressed in the poor prognosis patients include known markers of ES like EWS breakpoint region 1 and beta 2 microglobulin, genes regulating the cell cycle like CDK2, E2F, RAF and MAPKs, and genes associated with invasion and metastasis like cadherin-11 and MTA1. The last two belong to subclusters 5 and 6, genes which were homogeneously expressed in all patients. Down-regulated genes in the poor prognosis patients, included tumor suppressor genes like FHIT and LLGL1, genes inducing apoptosis like TNFRSF12, TGFB1, CASP10 and TP63 and inhibitors of angiogenesis like IFIT1 and IRF2.

Two genes that were significantly over expressed in the poor prognosis signature group ($p<0.01$) are of particular interest; both are associated with invasion and metastasis. The first one is cadherin11 (OB-cadherin), a homophilic calcium-dependent cell adhesion molecule, and the second is MTA1, tumor metastasis-associated gene. Cadherins modulate calcium ion-dependent cell-cell adhesion and are important in cell aggregation, migration and sorting. Defective cell-

cell and cell-matrix adhesion are among the hallmarks of cancer. Disruption of the cadherin-catenin complex has been demonstrated in carcinomas arising in several tissues including prostate, gastric and breast carcinomas, and has been correlated with various pathologic and clinical features, such as tumor differentiation, proliferation and a poor patient prognosis.

The MTA1 gene is a novel, highly conserved gene that encodes a nuclear protein product. Examination of the MTA1 protein suggests that it is a histone deacetylase and may serve multiple functions in cellular signaling, chromosome remodeling and transcription processes that are important in the progression, invasion and growth of metastatic cells. The gene has been found to be over-expressed in a variety of human cell lines (breast, ovarian, lung, gastric and colorectal) and cancerous tissues (breast, esophageal, colorectal, gastric and pancreatic cancer).

To validate the microarray data, these two over-expressed genes were analyzed in further detail using reverse transcriptase - quantitative Real Time PCR (RQ-PCR). Microarray-based expression and RQ-PCR based expression data correlated significantly (Fig. *2a* and *b)*. The mean log expression value of the poor prognosis signature group is significantly higher than that of the good prognosis signature group for both genes, cadherin-11 and MTA1, P=0.024 and P=0.003, respectively.

Six metastases from localized patients who progressed were further tested, using the unsupervised learning methodology, whether the poor and good prognosis signature set of genes can classify metastatic tissues to one of the prognostic groups, or as a distinct group.

While specific embodiments of the invention have been described for the purpose of illustration, it will be understood that the invention may be carried out in practice by skilled persons with many modifications, variations and adaptations, without departing from its spirit or exceeding the scope of the claims.

Claims

1. A method for assessing the prognosis of Ewing's Sarcoma (ES) patients comprising determining the expression pattern of a defined set of genes in tumor material obtained from said patients, and assigning said expression pattern to either a good prognosis or poor prognosis group.

2. The method according to claim 1, wherein the expression pattern of the aforementioned defined set of genes is determined by means of a technique selected from the group consisting of nucleic acid hybridization, semi-quantitative RT-PCR, quantitative real time RT-PCR, immunohistochemistry and ELISA.

3. The method according to claim 2, wherein the expression pattern of the aforementioned defined set of genes is determined by means of a nucleic acid hybridization technique.

4. The method according to claim 3, wherein the nucleic acid hybridization technique comprises the steps of extracting total RNA from the ES-patient tumor material, generating double-stranded cDNA from said total RNA, performing *in vitro* transcription of said cDNA, labeling the RNA transcript obtained thereby, hybridization of said RNA transcript to a solid-state human genome microarray.

5. The method according to claim 1, wherein the assignment of the gene expression pattern to one of the good or poor prognosis groups is performed by means of a hierarchical clustering technique.

6. The method according to claim 1, wherein the defined set of genes comprises genes selected from a group of 818 genes listed in Table 1, hereinabove.

7. The method according to claim 6, wherein the defined set of genes consists of between 1 and 100 genes selected from the group of 818 genes.

8. The method according to claim 6, wherein the defined set of genes consists of between 101 and 200 genes selected from the group of 818 genes.

9. The method according to claim 6, wherein the defined set of genes consists of between 201 and 300 genes selected from the group of 818 genes.

10. The method according to claim 6, wherein the defined set of genes consists of between 301 and 400 genes selected from the group of 818 genes.

11. The method according to claim 6, wherein the defined set of genes consists of between 401 and 500 genes selected from the group of 818 genes.

12. The method according to claim 6, wherein the defined set of genes consists of between 501 and 600 genes selected from the group of 818 genes.

13. The method according to claim 6, wherein the defined set of genes consists of between 601 and 700 genes selected from the group of 818 genes.

14. The method according to claim 6, wherein the defined set of genes consists of between 701 and 818 genes selected from the group of 818 genes.

15. A solid-state nucleic acid microarray comprising at least two nucleic acids affixed to a substrate, wherein each of said at least two nucleic acids consists of a partial sequence of one of the genes present in the group of 818 genes listed in Table 1, hereinabove.

16. The solid-state nucleic acid microarray according to claim 15 comprising 818 nucleic acid sequences, wherein each of said sequences consists of a partial sequence of one of the 818 genes listed in Table 1, hereinabove.

17. The solid-state nucleic acid microarray according to claim 15 further comprising one or more control nucleic acid sequences.

18. A kit comprising a solid-state nucleic acid microarray according to claim 15, together with an instruction sheet.

Fig. 1a

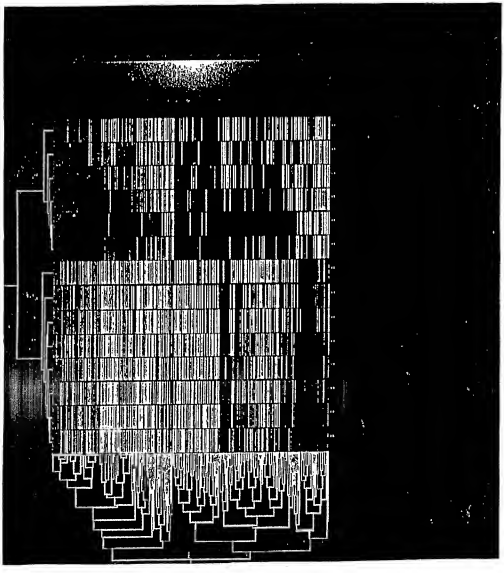


Fig 1b

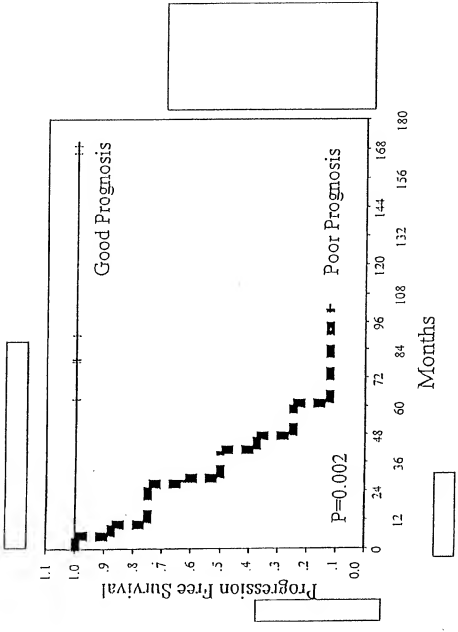

Progression Free Survival
1.1
1.0
.9
.8
.7
.6
.5
.4
.3
.2
.1
0.0
0
12
24
36
48
60
72
84
96
108
120
132
144
156
168
180
Months
Good Prognosis
Poor Prognosis
P=0.002

Fig. 1c

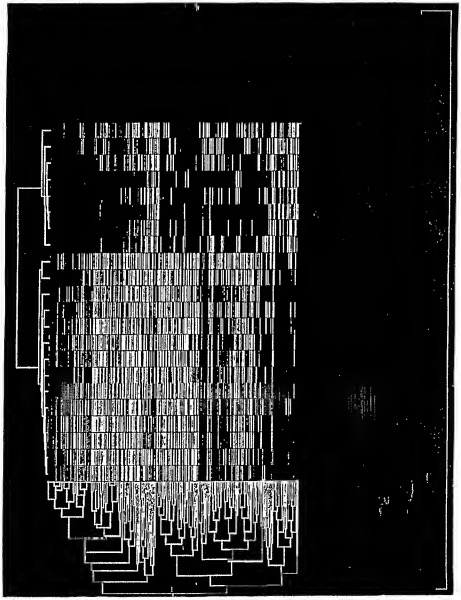
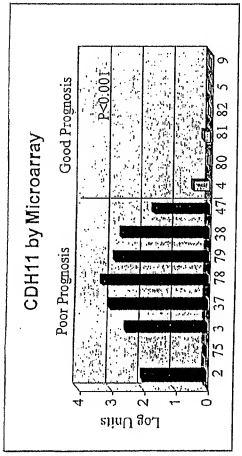


Fig. 2a


CDH11 by Microarray
Poor Prognosis
Good Prognosis
P<0.001
Log Units
0
1
2
3
4
2 75 3 37 78 79 38 47 4 80 81 82 5 9

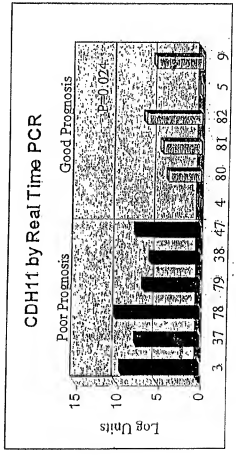

CDH11 by Real Time PCR
Poor Prognosis
Good Prognosis
P=0.02
Log Units
0
5
10
15
3 37 78 79 38 47 4 80 81 82 5 9

Fig. 2b